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STUDY OF TETRACYCLINE INDUCED ALTERATION IN ASCORBIC ACID CONTENTS IN FRESHWATER BIVALVES, *LAMELLIDENS CORRIANUS* (LEA) AND *PARREYSIA CYLINDRICA* (ANNANDALE AND PRASHAD)

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ABSTRACT
After exposure to lethal and sublethal concentrations of tetracycline, one of the broad spectrum antibiotics, the vitamin C level showed the depletion in various tissues except in gill and foot of experimental models, the freshwater bivalve, *L. corrianus* and *P. cylindrica*. The bivalves were exposed to acute dose of tetracycline ($LC_{50/2}$) 369.10PPM up to 96 hours and chronic ($LC_{50/10}$) 72.83 PPM up to 21 days. The ascorbic acid contents were estimated in mantle, gill, foot, testis, ovary, whole body and digestive gland after 24 and 96h of acute and 7, 14 and 21 days of chronic exposure. A lethal and sublethal concentration of tetracycline induced severe, progressive perturbations in tissues after 96 hrs and 21 days. The level of ascorbic acid showed almost an overall decrease in different tissues but mainly in gill and mantle on lethal and sublethal exposures. In *L. corrianus* the decrease in ascorbic acid was 22.22 % and 49.99% after 96 hrs and 21 days exposures respectively and in *P. cylindrica* 39.99% decrease after lethal dose induction of tetracycline. The foot and gill in case of *P. Cylindrical* showed increased ascorbic acid contents on lethal exposure and the rest tissues showed depleted level in the ascorbic acid contents. In the foot of *P. cylindrical* the level inclined up to 12.3% and 27.39 % when exposed to lethal and sublethal concentration and in gill it was 16.12% after exposure of lethal dose.

INTRODUCTION

The biochemical changes occurring in the body gives first indication of stress. During any stress, to overcome the altered situation extra energy is needed. The biochemical composition varies according to seasonal changes, environmental factors (temperature, salinity), starvation and contamination of pollutants due to different anthropogenic activities.

The bivalves resist against such unwanted conditions by its own way and try to minimize the effect of the altered situation by removing the toxicant or made it simple by biotransformation. Effects of toxicants result from their interaction with certain receptors of the organisms. Thus the impact of toxicant is exerted not only on cell but also on the cell content. Different toxicants affect the metabolic activities which are expressed in terms of different changes that occurred in bivalve (Abel, 1974; Langston, 1986; Lumet *et al*., 2000). Ascorbic acid, being important constituent in cellular metabolism, the interactions of the bimolecular gives proper idea of toxicant stress and its effect.

From the time ancient demand for the protein rich food, being fulfilled by fishes, shellfishes, bivalves and oyster like sources, as it provides many of the nutrient elements. The study of these nutrients is possible by the biochemical analysis of the different bimolecular in general and with the altering induced conditions in exposed animals in particular.

For different physiological acts vitamins are essential, although required in trace amount. Most of the animals can synthesize this water soluble vitamin by their own, but not the human being. Hence in case of human beings it is introduced through the diet.

Ascorbic acid acts as an essential factor for normal growth in rainbow trout, *Salmo gairdneri* (Halver *et al*., 1969; Tucker, 1983; Tucker and Halver, 1986). In terrestrial animals the dietary ascorbic acid has role in the host defense systems. Ascorbic acid influences various parameters of immunocompetence in the guinea pig (Thurman and Goldstein, 1979). Though the complete prevention of viral infection is not possible, high doses of ascorbic acid reduces potency of the viral diseases (Murata, 1975). The accumulation of ascorbic acid at the site of wound healing was found by Gould (1963). Interferons get enhanced in circulatory system after ascorbic acid ingestion through diet (Siegel, 1974). Lymphoidal tissue regeneration and their differentiation occurred under the influence of ascorbic acid (Dieler and Breitenbach, 1971). Siddique (1967) found the increase in ascorbic acid in liver, gonads and serum of *Ophiocephalus punctatus* with increase in temperature.

Thus ascorbic acid has a central position in curing the impaired condition occurred by the pathogenic attack and resists against the diseases in organisms. The impact of tetracycline on ascorbic acid content was studied in bivalves, *L. corrianus* and *P. cylindrica* in the present study because in artificial pearl culture during postoperative care bivalves are exposed to certain antibiotic treatment as there are more chances of bivalve mortality. Use of antibiotics reduces the rate of mortality.

Similarly in culture, to reduce the mortality rate of larvae of bivalves and oysters, antibiotics are used. So far, the side effects and toxic effects of antibiotics on the bivalves, oysters and other invertebrates are not yet studied.

MATERIALS AND METHODS

The freshwater bivalves, *L. corrianus* and *P. cylindrica* were collected from Girna dam, Dist: Nasik, M.S. The animals were acclimatized to laboratory conditions for 4 days prior to experimentation. During experimentation only those animals showing movements and in apparent good health, were used for investigation. The animals were divided into five groups, two for acute and two for chronic exposures of tetracycline and one group was maintained as control in each case.

a) Acute exposure to Tetracycline

The healthy bivalves, *Lamellidens corrianus* were exposed to acute treatment ($LC_{50/2}$) of tetracycline 369.10PPM, while *Parreysia cylindrica* were exposed to tetracycline 166.54PPM up to 96 hrs.

b) Chronic exposure to Tetracycline

The acclimatized *L. corrianus* were exposed to ($LC_{50/10}$) concentration of tetracycline 73.82 PPM while *P. cylindrica* were exposed to chronic concentration of tetracycline 33.30 PPM up to 21 days.

During exposure period, no special food was provided and the water with required concentration of tetracycline was changed daily in the experimental set. Control set was provided with dechlorinated water only without addition of tetracycline. After 24 and 96h of acute exposure and after every 7, 14 and 21 days of chronic exposure, the mantle, gill, foot, testis, ovary, digestive gland and the whole flesh were isolated, blotted to remove excess water and dried in oven at 80ºC till constant weight was obtained. All tissues were ground separately into fine powder from which ascorbic acid contents were estimated. Ascorbic acid content was estimated by using Hydrazine reagent by the method as given by Roe (1967). The calibration curves were drawn by plotting concentrations of standard against optical density to determine the corresponding value of ascorbic acid content from tissues after acute and chronic exposure to tetracycline. The results were expressed in mg per 100mg of dry tissue. The % variations were also calculated to find out the antibiotic induced stress to the biochemical substances undertaken for study and the test of significance was applied.

RESULTS AND DISCUSSION

The maximum ascorbic acid content was observed in mantle. There was a marked decrease in ascorbic acid contents in almost all tissues of *L. corrianus* and *P. cylindrica* after acute and chronic exposures. The significant effect of tetracycline observed in gills of *L. corrianus* was 49.99 % and the reverse trend was shown by the whole body 33.33 % increase in ascorbic acid contents after sublethal exposure of tetracycline (Table 1).

After 96h of lethal exposure to tetracycline showed incline pattern of ascorbic acid content in gill and foot 16.12% and 12.30 % respectively while sublethal exposure increased the contents in foot up to 27.39 % after 21 days in *P. cylindrica*. The rest of the tissues depressed the contents at different proportions as compared to the control. The overall effect as increase or decrease in ascorbic acid contents was significant at $P < 0.001$, $P < 0.01$ or $P < 0.05$ level for acute and chronic exposure while was non-significant in some cases (Table 2). The antioxidant role of ascorbic acid is a well-known phenomenon, which protects the tissues from the superoxide radical generated due to different toxicological effects. Changes in the environment cause alteration in the ascorbic acid content.

The varied functions of the ascorbic acid make it dynamic. Any alteration in the surrounding water due to the contamination of water also alters ascorbic acid contents.

Different pollutant stress has its impact on the concentration of ascorbic acid (Ali *et al*., 1983; Bhusari, 1987). Ascorbic acid contents increase during stress (Rao and Chinoy, 1986) and after metal intoxication indicating its role in detoxification process.

The curing response against methyl mercury damage was seen in the reproductive organs of guinea pig after ascorbate administration (Rao *et al*., 1994). Seymour (1981, b) reported that the levels of ascorbic acid in the ovaries of maturing crucian carp, *Carassius carassius* decreased after injection of pituitary extract. Wedemeyer (1969) observed that the stress-induced release of cortisol occurred concomitant with a decrease in the ascorbic acid in the kidney of salmonids.

In higher animals (vertebrates) the reduced exogenous requirement of ascorbic acid may be a result of its lower need for biochemical functions with age or an increased storage capacity combined with more efficient endogenous reuse. Jadhav *et al*. (1996) showed a decreased level of ascorbic acid content after pesticidal stress in *Corbicula striatella*. Waykar (2000) reported a decrease in the ascorbic acid level in various tissues of *Parreysia cylindrica* on exposure to pesticide.

Ascorbate protected the binding sites of the receptors and making the metal forms insoluble (Scheuhanner and Cherian, 1985). The rate of growth retardation caused by toxic metals was minimized by the ascorbate administration (Hill, 1979).

Clarkson *et al*. (1988) and Rao *et al*. (1994) reported that the oxygen radical formed due to methyl mercury forms reactive oxygen intermediates with ascorbic acid. Daine *et al*. (1994) showed the recovery from chromium intoxication by ascorbic acid treatment. Sometimes vitamin C and vitamin E acts in combination for detoxification (Chan, 1993; Meister, 1994). Mahajan and Zambare (2001) found that the reduction in protein depletion due to $CuSO_4$ and $HgCl_2$ was recovered by ascorbate treatment in *Corbicula striatella*.

Mouse peritoneal macrophages when elicited by the antioxidant ascorbic acid have been found to be significantly stimulatory, exhibiting significant enhancement in protein content, lysosomal acid hydrolase levels and capability to phagocytise (Agrawal *et al*., 2003). The ascorbic acid supply may boost the macrophage activity, helping to remove intracellular free irritant. These results indicate the positive role of ascorbic acid in toxicant stress.

The depleted level of ascorbic acid is a vivid response against tetracycline to cope up the toxic stress caused by exposure to

Table 1: Impact of Tetracycline on ascorbic acid content of *Lamellidens corrianus* after acute and chronic exposure

Tissues	24 h Control	Tetra	96 h Control	Tetra	7 d Control
M	1.5644 ± 0.2332	1.5111 ± 0.1716-3.409NS	1.5822 ± 0.0817	1.4222 + 0.105.-10.112**	0.9066 ± 0.1111
G	0.7288 ± 0.1166	0.5969 ± 0.0290-18.102NS	0.4800 ± 0.0101	0.3733 ± 0.0048-22.222***	0.2133 ± 0.0059
F	0.4266 ± 0.0817	0.3856 ± 0.0767-9.615***	0.9244 ± 0.0220	0.6933 ± 0.000-24.999***	0.6044 ± 0.0288
O	0.3555 ± 0.0101	0.2240 ± 0.0503-36.999**	0.3911 ± 0.0148	0.2371 ± 0.0767-39.363*	0.4266 ± 0.0290
T	0.3656 ± 0.0872	0.3200 ± 0.0817-14.268*	0.5155 ± 0.0503	0.4088 ± 0.0267-20.689**	0.5155 ± 0.0101
WB	0.3911 ± 0.0817	0.2844 ± 0.0105-27.272NS	0.106 ± 0.0059	0.071 ± 0.0148-33.333**	0.6755 ± 0.0675
DG	0.3555 ± 0.0503	0.3022 ± 0.0101-15.00NS	0.5155 ± 0.0477	0.3911 ± 0.0675-24.137**	0.2311 ± 0.0089

Table 1: Cont.....

Tissues	Tetra	14 d Control	Tetra	21 d Control	Tetra
M	0.7085 ± 0.0817-13.333*	1.2977 ± 0.1332	1.0204 ± 0.0817-21.369**	1.0341 ± 0.0503	0.7822 ± 0.0288-32.211***
G	0.1792 ± 0.000-15.999***	0.3022 ± 0.0817	0.1955 ± 0.0288-35.294*	0.4444 ± 0.0503	0.2222 ± 0.0059-49.999**
F	0.5511 ± 0.0290-8.823***	0.3200 ± 0.0089	0.2666 ± 0.0377-16.666*	0.2666 ± 0.02908	0.2133 ± 0.0014-20.00*
O	0.3164 ± 0.0267-25.833***	1.440 ± 0.0886	0.8938 ± 0.0288-37.925***	0.4800 ± 0.0288	0.2542 ± 0.0817-47.037**
T	0.3555 ± 0.0233-6.896***	0.6724 ± 0.0133	0.5688 ± 0.0503-18.200*	0.4622 ± 0.0290	0.3555 ± 0.000-23.076**
WB	0.6933 ± 0.0501 + 2.631NS	0.3733 ± 0.0101	0.3929 ± 0.0059 + 5.263**	0.3377 ± 0.0171	0.4503 ± 0.0050 + 33.33***
DG	0.1777 ± 0.0059-23.076***	0.3377 ± 0.0290	0.2311 ± 0.0101-31.578**	0.4622 ± 0.0220	0.2631 ± 0.0767-43.076**

M = Mantle; G = Gill; F = Foot; O = Ovary; T = Testis; WB = Whole body; DG = Digestive gland; Values are expressed as mg/100mg dry weight of tissue. ± indicates standard deviation of three independent replications; + or - indicates % variation over control. Significance: * $p < 0.05$; ** $p < 0.01$; *** p 0.001; NS = Non-significant

Table 2: Impact of Tetracycline on ascorbic acid content of *Parreysia cylindrica* after acute and chronic exposure

Tissues	24 h Control	Tetra	96 h Control	Tetra	7 d Control
M	1.208 ± 0.1332	0.888 ± 0.0267-26.470**	1.76 ± 0.377	1.230 ± 0.288.-30.080*	1.3866 ± 0.1716
G	0.6933 ± 0.0817	0.746 ± 0.0322 + 7.692NS	0.551 ± 0.0675	0.64 ± 0.0255 + 16.129*	0.7111 ± 0.1332
F	0.6044 ± 0.0872	0.3911 ± 0.0769-35.294***	1.155 ± 0.0872	1.297 ± 0.0355 + 12.307*	0.5866 ± 0.0872
O	0.2133 ± 0.0553	0.1807 ± 0.0290-15.278NS	0.302 ± 0.0502	0.220 ± 0.077-27.058*	0.4622 ± 0.0101
T	0.4622 ± 0.0503	0.4050 ± 0.0486-12.368***	0.502 ± 0.0288	0.399 ± 0.0503-20.528**	0.800 ± 0.20
WB	0.6755 ± 0.0872	0.3911 ± 0.0503-42.105**	0.106 ± 0.0050	0.053 ± 0.0001-50.0***	0.7642 ± 0.0293
DG	0.3733 ± 0.08728	0.3377 ± 0.0581-9.523NS	0.3377 ± 0.0293	0.3200 ± 0.0288-5.263**	0.3200 ± 0.0769

Table 2: Cont.....

Tissues	Tetra	14 d Control	Tetra	21 d Control	Tetra
M	1.28 ± 0.2880-7.692NS	1.315 ± 0.0817	1.048 ± 0.0504-20.270**	1.28 ± 0.1332	0.8177 ± 0.0503-36.311**
G	0.521 ± 0.0288-26.666*	0.4266 ± 0.0675	0.3022 ± 0.0059-29.166*	0.5333 ± 0.0220	0.320 ± 0.0817-39.999*
F	0.675 ± 0.05817 + 15.151NS	0.3733 ± 0.0290	0.4382 ± 0.0101 + 17.391***	0.4088 ± 0.02936	0.5208 ± 0.000 + 27.391***
O	0.3911 ± 0.0089-15.384***	1.244 ± 0.0767	0.9422 ± 0.2900-24.285**	0.5511 ± 0.05817	0.3520 ± 0.0293-36.129**
T	0.7686 ± 0.0581-3.921NS	0.9066 ± 0.0293	0.7978 ± 0.0501-12.00**	0.4444 ± 0.0288	0.3555 ± 0.0101-19.999**
WB	0.711 ± 0.0288-6.976***	0.533 ± 0.0101	0.4622 ± 0.0290-13.333**	0.6755 ± 0.0501	0.5333 ± 0.0290-21.052**
DG	0.2133 ± 0.0050-33.333***	0.3911 ± 0.0029	0.2666 ± 0.00-31.818***	0.9599 ± 0.0293	0.7075 ± 0.0767-26.296**

M = Mantle; G = Gill; F = Foot; O = Ovary; T = Testis; WB = Whole body; DG = Digestive gland; Values are expressed as mg/100mg dry weight of tissue. ± indicates standard deviation of three independent replications; + or - indicates % variation over control. Significance: * $p < 0.05$; ** $p < 0.01$; *** p 0.001; NS = Non-significant

antibiotics. The changed level of ascorbate reflects the great interaction among the biomolecules present in the cell cited in the present paper.

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